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Power Reagents™, Power Cloning™, Power Cell-free™, Power PCR™ and Open Cell-Factory™: Radegen Biotechnology's core product lineup.

By Fernando Andrade, M.S., Founder, CEO and CSO @ Radegen Biotechnology Skunkworks Division.

Power Reagents™ are a new type of molecular reagent produced from cell-free microbial lysates. Microbial cell-free lysates are generated from proprietary strains of microbial species like *Pseudomonas putida,* and *Escherichia coli* that are genetically engineered to heterologously over express specific enzymes of interest. During reagent production, the cellular chassis that carries the reagent protein payload is permeated and preserved using a proprietary Freeze-lysis procedure. Upon rehydration, the frozen protein vessel shatters and the contents are released for application as a reagent. This process is a central highlight of Radegen Biotechnology's low-consumable manufacturing processes and helps make this reagent class an environmentally friendly option with robust performance that is inherently as good or better than the competition. For many reagent applications, the protein background naturally found in cell-free lysate provides beneficial assistance.

Radegen Biotechnology™ is an Open Source Synthetic Molecular Biology company and we employ the use of two critical Open Source resources for the rational design of our revolutionary product lineup OpenBioeconomy.com and the following two articles: 2018. Cellular reagents for diagnostics and synthetic biology. PLoS One. Aug 15;13(8):e0201681. PMID: 30110361. (CC BY 4.0) and 2021. Producing molecular biology reagents without purification. PloS One. Jun 1;16(6):e0252507. PMID: 34061896.

Power Reagents™ is Radegen Biotechnology's proprietary and commercial name for our version of Cellular Reagents. **Power Reagents™** are fundamentally different from Cellular Reagents since we process our reagents to meet commercial standards of performance by increasing the concentration of protein of interest, use state-of-the-art buffers to maximize enzyme performance and prepare the reagent shifor long term cryostorage.

Power Reagents™ are thus a new and exciting class of reagents that for application purposes is better than pure enzyme reagents for specific applications for various reasons including product performance and cost.

- **PCR**: DNA binding proteins that are involved in DNA replication bind to DNA during PCR and assist in amplification, increasing the overall efficiency and sensitivity of a reaction. Many high-performance PCR reagents incorporate such peptides to provide this very function.
- **DNA Assembly and Cloning**: The natural protein machinery that mediates DNA replication/repair and homologous recombination i*n vivo* can be harnessed to assemble novel DNA constructs via a process termed cloning by homology-directed repair. Assemblyase™ is our proprietary cell lysates that powers all of Radegen Biotechnology's cloning technology. The system also incorporates cloning plasmids containing **PpuOriC™** an origin that are replicates fully circularized plasmids *in vitro* by the chromosomal replication/repair machinery, producing high transformation efficiencies in *E. coli* of the desired construct.

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- **Cell-free Protein Synthesis**: The natural protein machinery that mediates chromosomal replication is harnessed in Radegen Biotechnology reagents to make a more efficient protein synthesis reagent. *In vitro* expression plasmids designed for use with **Xpressase™** contain a **PpuOriC™** sequence that reacts with the chromosomal replication machinery, replicates the plasmid during protein synthesis, enriching the reaction with the template, a process that leads to a higher protein yield overall with a lower input DNA requirement.

A description the Power Reagent™ Lineup

1. **Power Cloning™ -** The first core competency of Radegen Biotechnology is cloning reagents. Our R&D has led to the development of cloning reagents that use *in vitro* homology directed repair for circularizing a cloning construct. The system is designed for use of any compatible plasmid or the included PpuOriC™ pRB plasmids. pRB plasmids contain the pUC high copy replication origin and are designed for propagation and maintenance in *E. coli* or *P. putida*. The PpuOriC™ feature of the pRB plasmid is for *in vitro* plasmid enrichment during a cloning reaction and does not interfere with plasmid function in *E. coli*.
    a. **Assemblyase™** - A bacterial cell free lysate reagent that is competent for homology directed repair of DNA. This system can either be produced from a RecA- strain of bacteria expressing the Lambda red operon or from a RecA+ bacterial strain. This system is specifically designed for assembling sense and antisense oligonuclotides with 15-40 nt of hybridizing homology shared between the terminal ends and the terminal ends of a linear destination plasmid.
    b. **Power Cloning T5™** - A cloning reagent formulated with T5 exonuclease in a RecA positive bacterial cell-free lysate background. This reagent is designed for the assembly of a single or multiple PCR fragments or gBlocks into any destination plasmid.

        >T5 Exonuclease
        MSKSWGKFIEEEEAEMASRRNLMIVDGTNLGFRFKHNNSKKPFASSYVSTIQSLAKSYSARTTIVLGDKGKSVFRLEHLPEYKGNRDEKYAQRTEEEK
        ALDEQFFEYLKDAFELCKTTFPTFTIRGVEADDMAAYIVKLIGHLYDHVWLISTDGDWDTLLTDKVSRFSFTTRREYHLRDMYEHHNVDDVEQFISLKA
        IMGDLGDNIRGVEGIGAKRGYNIIREFGNVLDIIDQLPLPGKQKYIQNLNASEELLFRNLILVDLPTYCVDAIAAVGQDVLDKFTKDILEIAEQ*

    c. **Power Cloning SapI™** - A cloning reagent formulated with SapI restriction enzyme and it's respective methyl transferase protein in a RecA positive bacterial cell-free lysate background. This reagent is designed for the assembly of a single or multiple SapI donor plasmids into a SapI destination plasmid.

        >SapI
        MRRLATQRREDAYKSNRDYQTVHEAQSLRVNSTDDDNLSLFLLKDISPREDSKNIVGFGGFVKPEIATTMALTLTTDIDKQIKSVPLSSNWNRISIVAK
        FASNPSVSITLGFDQTPWVDFWGINSDDIGLSFVSDAVPLEMSMIDSIHIAPETLYLDHSSACLLDIDPVESTRFKTGHGDPLSLKKCSYCGRLLPIDLE
        RPGKLSFHKHRAKITNHQNECRSCKKWRINNSFNPMRTIDQLNESALITRERKIFLQEPEILQEIKDRTGAGLKSQVWERFHRKCFNCRKDLKLSEVQ
        LDHTRPLAYLWPIDEHATCLCAQCNNTKKDRFPVDFYSEQQIRELSDICGLPYQDLCARSLNLDQLDRIERNIAEFSKEWDVRTFASTARRISEVYPAR
        DLFETLKKESESAYNKIIEKLKERPDALLDEALPLD*
        >SapI.M1
        MLETLELVNKIAEFQRKLPYTQDDYKSRSWGHPLHSLCSYQGKLKPSLAHWLVKTFSPEGGTVLDPMGGVGTIAFEAALTGRVGITNDKSPLAATVTA
        GKLAPSSILEAEEAIGRLAEDIESVDLSAADYEAANFGLNARVSDYYHPDTLKEILRARRIFSERREAYPAFVWASLLHVLHGNRPYALSRISHPITPFN
        PSGVAEYRSVVEKIAHRARLALRNPLPEAFTSGAAIEGDFRDLSEHINEPVDAIITSPPFMGMRFDRPNWLRLWFCGWDAEDFWTTSLGFLERHQVK
        SRDSYIDFFEMSIKTLKQDGLLVMHLGSGGKKNLVNDLKSLAVPLFELAGEVIEDVDDHQTHGIRDRGLTTKHHLLFFKPA*
        >SapI.M2
        MSVDAPSPARRRGQAATSGRGTNENRLPIDLGVTFRDNRNRPVHSWYPYVEGFSAAYVEGVLAPYNGHNVAVYDPFGGSGTLQSTASWLGINSFYS
        EVNPFMRFVAEAKVNATLKAAQNKDVFRCAAKEFLDMLSEKELAHRGRSVDLSQYYSAFPGRDFFEEEHIRQLLAACDAARLIGSDYAWVRQLLLLA
        CAANAVHSSNMTRRADLRRRRQNEYINRKVDVARFISDTVQAMLDDVEQVPFGAVASHYVSDDCRDLPSRYIDCFDIAITSPPYLNGTNYFRNTKIEL
        WLLGFLSHESELPKFCREAITAGINNVSGNKALDHHFDVVEDVATKLDDVAPDRRIPKLVRHYFSDMYEVLTSVRSSLRLGGRFILDIGDSKFYGVHVP
        VDRILVELGKQVGFQLHQDVVIARRHSRDKTPLVQVELEFRKA*

    d. **Power Cloning T5-SapI™** - A cloning reagent formulated with SapI restriction enzyme, its respective methyl transferase protein, T5 exonuclease in a RecA positive bacterial cell-free lysate background. . This reagent is designed for the assembly of a single or multiple SapI donor plasmids into any destination plasmid.

2. **Power PCR™ -** Cloning reagents produced in a *P. putida*, EM383. Overexpression of polymerases and accessory proteins in cell lysates from single strains of EM383 are combined to produce functional reagents. This class of polymerase reagent also contains natural proofreading and DNA binding proteins that improve fidelity and output.
    a. Power Vent™ - a polymerase reagent produced from Psp polymerase first isolated from Pyrococcus species. This polymerase is heat stable at 95C for 23 hours and is ideal for high GC PCR.

b. Power Fusion - a polymerase reagent produced from a synthetic chimeric polymerase produced from as fusion between the Sso7D domain from Sulfolobus solfataricus and the polymerase first isolated from *Pyrococcus woesei.* This hybrid polymerase has a fidelity rate at least 50x higher than Taq.
c. Power+Start - a polymerase reagent produced from the DNA polymerase Thermus thermophilus and formulated with a Tth specific aptamer that specifically inhibits PCR outside of PCR temp conditions.
d. Proteins uses in Power PCR™

> Tth+Start Polymerase™
MHHHHHHSSGLVPRGSGMKETAAAKFERQHMDSPDLGTDDDDKMEAMLPLFEPKGRVLLVDGHHLAYRTFFALKGLTTSRGEPVQAVYGFAKSLLK
A LKEDGYKAVFVVFDAKAPSFRHEAYEAYKAGRAPTPEDFPRQLALIKELVDLLGFTRLEVPGYEADDVLATLAKKAEKEGYEVRILTADRDLYQLVS
DRVAVLHPEGHLITPEWLWEKYGLRPEQWVDFRALVGDPSDNLPGVKGIGEKTALKLLKEWGSLENLLKNLDRVKPENVREKIKAHLEDLRLSLELS
RVRTDLPLEVDLAQGREPDREGLRAFLERLEFGSLLHEFGLLEAPAPLEEAPWPPPEGAFVGFVLSRPEPMWAELKALAACRDGRVHRAADPLAGLK
DLKEVRGLLAKDLAVLASREGLDLVPGDDPMLLAYLLDPSNTTPEGVARRYGGEWTEDAAHRALLSERLHRNLLKRLEGEEKLLWLYHEVEKPLSRV
LAHMEATGVRLDVAYLQALSLELAEEIRRLEEEVFRLAGHPFNLNSRDQLERVLFDELRLPALGKTQKTGKRSTSAAVLEALREAHPIVEKILQHRE
LTKLKNTYVDPLPSLVHPRTGRLHTRFNQTATATGRLSSSDPNLQNIPVRTPLGQRIRRAFVAEAGWALVALDYSQIELRVLAHLSGDENLIRVFQE
GKDIHTQTASWMFGVPPEAVDPLMRRAAKTVNFGVLYGMSAHRLSQELAIPYEEAVAFIERYFQSFPKVRAWIEKTLEEGRKRGYVETLFGRRRYVP
DLNARVKSVREAAERMAFNMPVQGTAADLMKLAMVKLFPRLREMGARMLLQVHDELLLEAPQARAEEVAALAKEAMEKAYPLAVPLEVEVGMGED
WL SAKG* > >StartTth Aptamer™ tggcggagcgatcatctcagagcattcttagcgttttgttcttgtgtatgatttgccggccaatgtacagtattggccggc

> Pwo Fusion Polymerase™
MHHHHHHSSGLVPRGSGMKETAAAKFERQHMDSPDLGTDDDDKMILDVDYITEEGKPVIRLFKKENGKFKIEHDRTFRPYIYALLRDDSKIEEVKKI
TGERHGKIVRIVDVEKVEKKFLGKPITVWKLYLEHPQDVPTIREKVREHPAVVDIFEYDIPFAKRYLIDKGLIPMEGEEELKILAFDIETLYHEGEE
FGKGPIIMISYADENEAKVITWKNIDLPYVEVVSSEREMIKRFLRIIREKDPDIIVTYNGDSFDFPYLAKRAEKLGIKLTIGRDGSEPKMQRIGDMT
AVEVKGRIHFDLYHVITRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWESGENLERVAKYSMEDAKATYELGKEFLPMEIQLSRLVGQPLWDVS
RSSTGNLVEWFLLRKAYERNEVAPNKPSEEEYQRRLRESYTGGFVKEPEKGLWENIVYLDFRALYPSIIITHNVSPDTLNLEGCKNYDIAPQVGHKF
CKDIPGFIPSLLGHLLEERQKIKTKMKETQDPIEKILLDYRQKAIKLLANSFYGYYGYAKARWYCKECAESVTAWGRKYIELVWKELEEKFGFKVLY
IDTDGLYATIPGGESEEIKKKALEFVKYINSKLPGLLELEYEGFYKRGFFVTKKRYAVIDEEGKVITRGLEIVRRDWSEIAKETQARVLETILKHGD
VEEAVRIVKEVIQKLANYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKKLAAKGVKIKPGMVIGYIVLRGDGPISNRAILAEEYDPKKHKYDAEYY
IENQVLPAVLRI

> Psp Vent Polymerase™
MHHHHHHSSGLVPRGSGMKETAAAKFERQHMDSPDLGTDDDDKMIIDADYITEDGKPIIRIFKKEKGEFKVEYDRTFRPYIYALLKDDSAIDEVKKI
TAERHGKIVRITEVEKVQKKFLGRPIEVWKLYLEHPQDVPAIREKIREHPAVVDIFEYDIPFAKRYLIDKGLTPMEGNEELTFLAVDIETLYHEGEE
FGKGPIIMISYADEEGAKVITWKSIDLPYVEVVSSEREMIKRLVKVIREKDPDVIITYNGDNFDFPYLLKRAEKLGIKLPLGRDNSEPKMQRMGDSL
AVEIKGRIHFDLFPVIRRTINLPTYTLEAVYEAIFGKSKEKVYAHEIAEAWETGKGLERVAKYSMEDAKVTFELGKEFFPMEAQLARLVGQPVWDVS
RSSTGNLVEWFLLRKAYERNELAPNKPDEREYERRLRESYEGGYVKEPEKGLWEGIVSLDFRSLYPSIIITHNVSPDTLNRENCKEYDVAPQVGHRF
CKDFPGFIPSLLGNLLEERQKIKKRMKESKDPVEKKLLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGRQYIDLVRRELESSGFKVLYI
DTDGLYATIPGAKPNEIKEKALKFVEYINSKLPGLLELEYEGFYARGFFVTKKKYALIDEEGKIVTRGLEIVRRDWSEIAKETQAKVLEAILKHGNV
DEAVKIVKEVTEKLSKYEIPPEKLVIYEQITRPLSEYKAIGPHVAVAKRLAAKGVKVKPGMVIGYIVLRGDGPISKRAIAIEEFDPKKHKYDAEYYI
ENQVLPAVERILRAFGYRKEDLRYQKTKQVGLGAWLKF*

> Pfu+ UTPase™
MHHHHHHSSGLVPRGSGMKETAAAKFERQHMDSPDLGTDDDDKMLHHVKLIYATKSRKLVGKKIVLAIPGSIAAVECVKLARELIRHGAEVHAVMS
E AATKIIHPYAMEFATGNPVITEITGFIEHVELAGEHENKADLILVCPATANTISKIACGIDDTPVTTVVTTAFPHIPIMIAPAMHETMYRHPIVREN
IERLKKLGVEFIGPRIEEGKAKVASIDEIVYRVIKKLHKKTLEGKRVLVTAGATREYIDPIRFITNASSGKMGVALAEEADFRGAEVTLIRTKGSVK
SFVENQIEVETVEEMLSAIENELRSKKYDVVIMAAAVSDFRPKIKAEGKIKSDRSITIELVPNPKIIDRIKEIQPNVFLVGFKAETSKEKLIEEGKR
QIERAKADLVVGNTLEAFGSEENQVVLIGRDFTKELPKMKKRELAERIWDEIEKLLS*

3. **Power Cell-free™ Xpressase™ -** a reagent class and a reagent, this bacterial cell-free lysate is specifically designed for cell-free biochemistry. Produced from a proprietary formulation of EM383 and BL21. The system is designed to work with any T7 promoter or Trc promoter expression cassette.
4. **Open Cell-factory™ - a competent cell product designed for every step of a cell engineering reaction including expression plasmid and cloning reagents, competent cells, and transformation reaction reagents. The product line includes competent cell/cloning reagent packages that include *E. coli* BL21, DH5aplha and *P. putida* EM383.**

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